International Journal of General Medicine

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ORIGINAL RESEARCH

Clinical Investigation of Hereditary and Acquired Thrombophilic Factors in Patients with Venous and Arterial Thromboembolism

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Background: The clinical relevance of thrombophilic laboratory factors, especially the "mild" ones, and the need for their screening is not generally recommended in venous (VTE) and/or arterial (ATE) thromboembolism.

Methods: Our aim was to investigate possible associations between comorbidities and 16 inherited/acquired "severe" and "mild" laboratory thrombophilic factors (detailed in introduction) in patients (n=348) with VTE/ATE without a serious trigger (high-risk surgical intervention, active cancer and/or chemo-radiotherapy). Cases with VTE/ATE were enrolled when the thrombotic event occurred under the age of 40, in case of positive family history, recurrent thromboembolism, idiopathic event or unusual location. Patients without a detailed thrombophilia screening or who suffered from both ATE/VTE were excluded to find potential distinct thrombosis type specific thrombophilic risks. The possible role of "mild" factor accumulation was also investigated in VTE (n=266).

Results: Elevation of factor VIII clotting activity was associated with VTE rather than ATE. Varicose veins together with postthrombotic syndrome were strongly related to several "mild" factors. Besides "severe" we found that the "mild" thrombophilic factors were also strongly associated with VTE/ATE. Comorbidities/conditions such as diabetes and smoking were generally associated with hyperlipidemia; moreover, both had a correlation with lipoprotein (a) in VTE. We also revealed an important contribution of "mild" factors in increasing trends of several types and localizations of VTE.

Conclusion: In summary, besides the "severe" thrombophilic factors, the "mild" ones also seem to play a non-negligible role in the manifestation of thrombosis, especially in combination. Therefore, an extended screening might be useful in the personalized recommendation of antithrombotic prophylaxis.

Keywords: thrombosis, hemostasis, thrombophilia, venous thromboembolism, arterial thromboembolism

Introduction

Thrombosis is a multi-etiological disease with several inherited and acquired risk factors both in the venous (VTE) and arterial (ATE) form.[1,2] Though numerous thrombophilic factors detected by laboratory methods are known, the extent of screening is not unequivocal in various clinical situations, especially in ATE. There is widespread consensus that untargeted thrombophilia screening has little relevance in an unselected population.[3,4] On the other hand, younger patients or cases with unprovoked/unusual site/recurrent thrombosis might be candidates for laboratory thrombophilia screening in VTE.[5,6] Several congenital and acquired factors and scoring systems have been suggested in arterial or venous risk assessment tools, although essential risk factors are sometimes lacking from the list and there are differences also in the nomenclature.[1,2,7,8]

Received: 24 April 2023
Accepted: 19 October 2023
Published: 22 November 2023

The "severe" laboratory thrombophilic factors (SFs) are more frequently associated with serious and recurrent thromboembolism, postthrombotic syndrome and thrombotic complications in unusual localizations in younger age when compared to "mild" thrombophilic factors (MFs) characterized mostly in VTE. Antithrombin (AT), protein C (PC), protein S (PS) deficiencies, lupus anticoagulant, factor V Leiden p.R2506Q (FVLeiden) and FII G20210A (FII) homozygous mutations are generally determined as severe, while the heterozygous mutations of FVLeiden and FII, elevated factor VIII clotting activity (FVIII:C), von Willebrand factor antigen (vWF:Ag), homocysteine, methylenetetrahydrofolate reductase (MTHFR) C677T homozygous or heterozygous mutations, lipoprotein (a) (Lp(a)), existence of anti-cardiolipin (aCL) antibodies or anti-beta2-glycoprotein I (aB2GPI) antibodies are usually defined as mild thrombophilia.[3,9,10]

The role of conventional ATE risk factors such as hyperlipidemia or diabetes mellitus in the pathogenesis of VTE is controversial. Smoking is a common severe ATE risk factor in general, but it also raises the risk of VTE occurrence.[11,12] It is a fact that overall risk of VTE/ATE in patients with autoimmune disease or with hypo/hyperthyroidism is higher than in the normal population.[13–15] Varicose veins and several triggers (eg, gravidity, puerperal period, trauma, infection, surgery, immobilization, long-distance travel, catheterization etc.) are also known to be predisposing provoking factors in VTE.[16,17]

Though not to the same extent, the pathogenic role of various thrombophilic lab factors have been established both in the venous and arterial side.[15–33] According to the literature, the combination or accumulation of investigated thrombophilic factors can enhance the risk of VTE.[34–37]

Hereby, we present our data in order to find possible correlations among thrombophilic factors and different types of VTE/ATE without a serious trigger. We defined a rationale scale of mild laboratory factors which - with relevant clinical data - might be part of an extended screening in VTE in the future.

Methods

Study Population and Setting

From 2011 until 2016, 479 patients' data were collected in the Centre. According to clinical judgement we specified the following clinical inclusion criteria: VTE/ATE under the age of 40, positive family history, recurrent thromboembolic events. In VTE: severe venous event without any provoking factors or deep vein thrombosis (DVT) in an unusual site, in ATE: special criteria were: traditional atherosclerotic risk assessment seemed to be incongruent with the severity of the thrombotic disease. Patients without a detailed thrombophilia screening, and those with serious thrombotic triggers (a high-risk surgical intervention, active cancer and/or chemo-radiotherapy) were excluded. 131 patients who suffered from both arterial and venous thromboembolic events (ATE - VTE) were also excluded in order to homogenize the VTE/ATE groups, so finally 348 cases were analyzed. Data were collected from the medical charts and via personal interviews. VTE patients were diagnosed with distal DVT, proximal DVT, pulmonary embolism (PE), pulmonary embolism associated with deep vein thrombosis at the same time (PE+DVT) or unusual site thrombosis as central retinal vein occlusion (CRVO), splanchnic, cerebral sinus or upper extremity DVT. ATE patients were diagnosed with anterior ischemic optic neuropathy or retinal artery thrombosis, acute myocardial infarction, transient ischemic attack, ischemic stroke or peripheral artery disease. The relatively high number of ophthalmologic patients among ATE patients was due to the collaboration between our Centre and the Department of Ophthalmology. VTE and ATE were diagnosed by using actual clinical criteria and guidelines.

Diseases and Conditions

We registered the age at the time of the first thrombotic event, positive family history (ie, one or more family members – not exclusively first-degree relatives who had suffered from VTE or ATE). We noted the following diseases and conditions as risk factors: smoking (at least 10 cigarettes/day), hyperlipidemia, autoimmune disease, hypo/hyperthyroidism, diabetes mellitus (impaired glucose tolerance or manifest disease), varicose veins and postthrombotic syndrome. Putative provoking factors such as pregnancy, postpartum period, use of contraceptive pills, trauma, catheterization, infection, surgery, long-distance travel and immobilization were also screened.

Laboratory Methods

The patients underwent thrombophilia screening including laboratory thrombophilic risk factors. At the time of blood collection for testing – except for genetic examination - patients were either free from anticoagulant therapy or were given low molecular weight heparin (LMWH) at least 10 days prior to blood being drawn. Blood was drawn into 0.109 mol/L citrated vacutainer tubes (Beckton Dickinson, Franklin Lakes, NJ, USA) from all patients who underwent hemostasis laboratory investigations. Plasma samples were prepared by centrifugation at 1500 *g* for 20 minutes at room temperature and investigated immediately or stored at −80°C until use. For plasma homocysteine measurement, EDTA-anticoagulated sample was collected and for the Lp(a) determination a native blood sample was drawn (Beckton Dickinson). The subsequent lab parameters were measured in the study population: AT, PC, PS, FVLeiden, FII polymorphism, vWF:Ag, FVIII:C, homocysteine, MTHFR, Lp(a), lupus anticoagulant, aCL antibodies, aB2GPI antibodies. The following were considered SF: FVLeiden homozygous mutation, FII homozygous mutation, AT deficiency (hereditary/acquired), PC deficiency (hereditary/acquired), PS deficiency (hereditary/acquired), lupus anticoagulant, while the MFs were: FVLeiden heterozygous mutation, FII heterozygous mutation, elevated FVIII:C, vWF:Ag or Lp(a) levels, aCL antibodies, aB2GPI antibodies and MTHFR homozygous/heterozygous mutations and elevated fasting homocysteine. Only patients without C-reactive protein elevation were tested for thrombophilia at least 8 weeks after any thromboembolic events. AT activity was measured by Innovance AT kit (Siemens, Marburg, Germany), PC and PS activity were measured by Protein C coag and Protein S Ac kits, respectively (Siemens) and free PS antigen was detected by Innovance free PS Ag assay from Siemens. AT antigen was measured by immunonephelometry (BN ProSpec System AT-III, Siemens). PC antigen was determined by ELISA (Diagnostica Stago, Asnieres, France). If PC, PS, AT activities and/or antigen levels were decreased in at least two independent and time distinct measurements, the molecular genetic investigations were carried out by Sanger sequencing. The protocols of molecular genetic investigations will be provided on request. If the patient was FVLeiden homozygous or heterozygous and had low PC, PS activities in the functional clotting assays, due to the known interference effect of FVLeiden, the diagnosis of inherited PC/PS deficiency was established only if causative mutations in their PROC and PROS1 genes were found. In patients with autoimmune disease who had a considerable decrease below the lower limit of normal PS/PC activity, if no mutation was found in the background, we suspected an acquired PS/PC deficiency. FVIII:C was measured by clotting assay using FVIII deficient plasma and activator (Pathromtin SL) from Siemens. vWF:Ag was tested by von Willebrand Antigen kit from Siemens. All these parameters were measured on a BCS-XP automated coagulometer (Siemens). Lupus anticoagulant was detected according to the recommendation of the ISTH, briefly, Diluted Russel viper venom screening and confirming tests and APTT sensitive to lupus anticoagulant were performed by reagents from Werfen (Barcelona, Spain) and from Diagnostica Stago (Asnieres, France), respectively. The tests were run on an ACL Top 550 automated coagulometer (Werfen). ACL and aB2GPI antibodies were tested by ELISA (Werfen). FVLeiden, FII and MTHFR mutations were assessed using routine PCR-based methods (real-time PCR followed by melting curve analysis, primers and protocols are provided upon request) on a LightCycler 480 instrument (Roche, Mannheim, Germany). Total plasma homocysteine concentration was measured on Abbott Architect analyzer (Abbott, Abbott Park, IL). Lp(a) was measured by routine method on a Roche clinical chemistry analyzer. All methods were carried out in accordance with relevant guidelines and regulations. The University of Debrecen institutional committee approved all experimental protocols. Informed consent was obtained from all subjects.

Statistical Analysis

We performed multivariate analysis by using R 3.4.4 statistical software (R Core Team, 2023). Age, as a continuous variable, was tested by Kolmogorov–Smirnov test in order to assess normality and showed normal distribution. Statistical differences in age between the different study groups were analyzed using a two-tailed unpaired Student's *t*-test. In order to evaluate non-random associations between two categorical variables, Fisher's exact test was performed, otherwise, between three or more categorical variables, we used Chi-squared test in order to analyze cross-tabulations. P values less than 0.05 were considered statistically significant.

In order to map the multifactorial associations between the components, we used the so-called Kulczynski similarity measure between laboratory thrombophilic factors and comorbidities/conditions by dividing the number of the total matches with the total number of the single matches as follows: a/(b+c), where "a" indicates total matches (mutual presence of factors and conditions); "b" and "c" represent single matches (presence of only factors or conditions).[38] Hence, the Kulczynski measure calculates the ratio of total matches to single matches taking 0 value if there is no mutual presence of the laboratory factors and comorbidities/conditions (hence a = 0 and the quotient is also 0) and it takes a positive value otherwise (a and (b+c) are both positive). First, we calculated all the Kulczynski measures for each factor and condition pairwise. In this way, we created a matrix of the Kulczynski measures where the rows of the matrix are the laboratory factors and the columns represent the comorbidities/conditions. The cumulative density function of the Kulczynski measures was also calculated from the sample and the 95% critical value was determined to assess statistically significant differences to these measures. The cumulative density function was calculated by summing up the relative frequencies of the individual Kulczynski measures and it was used to describe how probable it is to get a certain (or lower) value for the Kulczynski measure. The 95% critical value means that 95% of the Kulczynski measures should be lower than this. If we exceed this critical value, our Kulczynski measure is more extreme and different to 0 (hence higher chance for a mutual presence of both factors and conditions). Therefore, we needed the critical value for the standard hypothesis testing. The distribution of Kulczynski measure was not normal therefore the standard formulas could not be used for estimating the confidence intervals for the mean value. We applied bootstrapping with 10,000 replicates using the boot package in R statistical software in order to calculate the confidence intervals of the mean.

In the next step we performed a Principal Component Analysis for this matrix. The purpose of this multifactorial analysis was to reduce the dimensionality of the large dataset into a smaller one by preserving as much variation of the values as possible. Therefore, the primary multidimensional connections could simply be revealed. Also, it graphically represented the Kulczynski similarity matrix in a two dimensional (biplot) graph and the study connections between the rows (factors) and columns (conditions) of the matrix. Principal Components (PrC)s are new variables that are computed by using a simple linear regression of the original factors. PCs as axes in a coordinate system are able to provide a better visualization of the differences between the observations. These PrCs can also separate the variables from each other, grouping them upon the strength and direction of their connections.

Table 1 represents the basic performance indicators (Kaiser-Meyer-Olkin /KMO/ and Bartlett test, explained variances) of the performed Principal Component Analyses. KMO test measures the strength of partial correlations compared to the total sum correlations. Partial correlation measures how factors explain each other pairwise without using the remaining factors (pairwise dependence). In case the KMO value is close to 0, the partial correlations are quite large which causes a problem for Principal Component Analysis. A KMO value close to 1 means the lack of pairwise dependence and a strong interrelationship between the studied factors, which is desirable for Principal Component Analysis. The cut-off value is 0.5 for the KMO test. Bartlett test is also designed to measure the strength of pairwise correlation between the factors, but in another way (in a matrix form). First, the pairwise correlation matrix is calculated and then compared to the identity matrix (its main diagonal is always 1 and elsewhere it contains only zeros). If the correlation matrix resembles the identity matrix, it means that factors are uncorrelated and not suitable for Principal Component Analysis.

Table 1 Summary of the Principal Component Analyses

Factor Groups	Explained Variance		Sum of the First Two PrCs	KMO	Bartlett Test	
	PrC1	PrC2			Chi2 (df)**	P
Localization of thromboses	43.44%	26.89%	70.33%	0.678	82.8 (28)	<0.001
Accompanied diseases	54.35%	35.27%	89.62%	0.855	119.4 (21)	<0.001

Note: **Chi-squared test.
Abbreviations: KMO, Kaiser-Meyer-Olkin test for measuring adequacy; PrC1, Principal Component 1; PrC2, Principal Component 2; df, degrees of freedom.

Each analysis was adequate and satisfied the minimum conditions (KMO exceeded 0.5) and the Bartlett test was also significant indicating that the data were appropriate for the analyses (pairwise correlation matrix differs from the identity matrix). The explained variance by the first PrC2 is larger than 70% and the PrC1 contributes to more than 50% of the explained variance.

All the Principal Component Analyses were performed by using the Varimax rotation to create more interpretable PrCs. For all the calculations R 3.4.4 was used with REdaS package for KMO and Bartlett tests of sampling adequacy and sphericity and prcomp function was used for calculating Principal Component Analysis.

Results

First, we compared the clinical characteristics of VTE patients (n=266)/ATE (n=82) in order to evaluate the study population and study settings.

In many conditions there were no differences between the two groups. Patients' mean age at first thrombosis was significantly lower in VTE than in ATE group. Smoking was more common in ATE, but recurrent events, provoking factors, varicose veins and postthrombotic syndrome were more frequent in the VTE group. The level of FVIII:C was high in both groups, but it was significantly higher in VTE than in ATE. However, after positive family history, hyperlipidemia, diabetes mellitus, autoimmune disease, or hypo/hyperthyroidism, all the other thrombophilic lab factors were also present in a large percentage in our selected patients without significant differences between the two groups. So except for FVIII:C, no other marked differences were found in laboratory thrombophilic risk factors or in examined comorbidities/conditions in VTE/ATE (Table 2).

Subsequently, we examined the presence of thrombophilic lab factors in connection with various locations of VTE (n=266). We also tested the correlation of comorbidities/conditions with VTE/ATE. Due to the low number of patients in ATE group, further statistical analyses were not performed.

The main idea was to create two new components (latent factors or PrCs) from the comorbidities or locations of VTE (based on the correlation matrix) and calculate the new component values for the thrombophilic lab factors. The so-called biplot (Figure 1) represents how new components were related to comorbidities and the component values of the lab factors. We can also see clusters of comorbidities (or locations of VTE) and lab factors and their relationship as well. For the sake of better visualization, we performed two analyses, one for the comorbidities and another one for the locations of VTE. We first present the results of the first Principal Component Analysis with respect to comorbidities (Figure 1).

Table 2 Clinical Characteristics of Patients with VTE/ATE

VTE (266 Patients)		ATE (82 Patients)	
Distribution of diagnoses			
Distal deep vein thrombosis	36%	Anterior ischemic optic neuropathy/retinal artery thrombosis	41%
Proximal deep vein thrombosis	32%	Transient ischemic attack	33%
Pulmonary embolism	26%	Peripheral artery disease	32%
Central retinal vein thrombosis	18%	Ischemic Stroke	29%
Upper extremity deep vein thrombosis	18%	Acute myocardial infarction	15%
Pulmonary embolism and deep vein thrombosis at the same time	11%		
Splanchnic vein thrombosis	6%		
Cerebral venous sinus thrombosis	3%		

(*Continued*)

Table 2 (Continued).

VTE (266 Patients)		ATE (82 Patients)	
Risk factors and comorbidities			
Age	53.38y±16.84		54.45y±15.38
Gender ♂	35%		50%*
Age at first thrombosis	39.77y±16.35		48.02y±14.99*
Smoking	12%		33%*
Recurrent events	41%		23%*
Provoking factor	44%		5%*
Varicose veins	16%		1%*
Postthrombotic syndrome	31%		0%*
Positive family history	56%		63%
Hyperlipidemia	61%		70%
Diabetes mellitus	15%		18%
Autoimmune disease	37%		28%
Hypo/hyperthyroidism	13%		13%
Laboratory factors			
Homocysteine↑	39%		51%
vWF:Ag↑	45%		33%
Lp(a)↑	31%		33%
FVIII:C↑	44%		28%*
MTHFR heterozygous	17%		26%
MTHFR homozygous	5%		3%
aCL antibodies	16%		13%
FVLeiden heterozygous	27%		20%
aB2GPI antibodies	9%		7%
FII heterozygous	13%		16%
PC deficiency (SF)	5%		7%
Lupus anticoagulant (SF)	5%		4%
PS deficiency (SF)	7%		3%
FVLeiden homozygous (SF)	3%		2%
AT deficiency (SF)	4%		2%
FII homozygous (SF)	1%		0%

Notes: *p<0.05. Antithrombin (AT) deficiency: AT activity <80%, Protein C (PC) deficiency: PC activity <70%, Protein S (PS) deficiency: PS activity <60%, factor VIII clotting activity (FVIII:C) ↑: FVIII:C >150%, von Willebrand factor antigen (vWF:Ag) ↑: vWF:Ag >160%, homocysteine ↑: homocysteine to > 12.5 µmol/L, lipoprotein(a) (Lp(a)) ↑: Lp(a) >300 mg/L, anti-cardiolipin antibodies: aCL IgG/IgM > 20 U/mL, anti-beta-2-glycoprotein I antibodies: aB2GPI antibodies IgG/IgM > 20 U/mL, FII: prothrombin G20210A polymorphism. It is to be noted that n=131 patents had combined thrombotic diseases.

Abbreviations: FVLeiden, factor V Leiden p.R2506Q mutation; MTHFR, methylenetetrahydrofolate reductase; SF, serious factor.

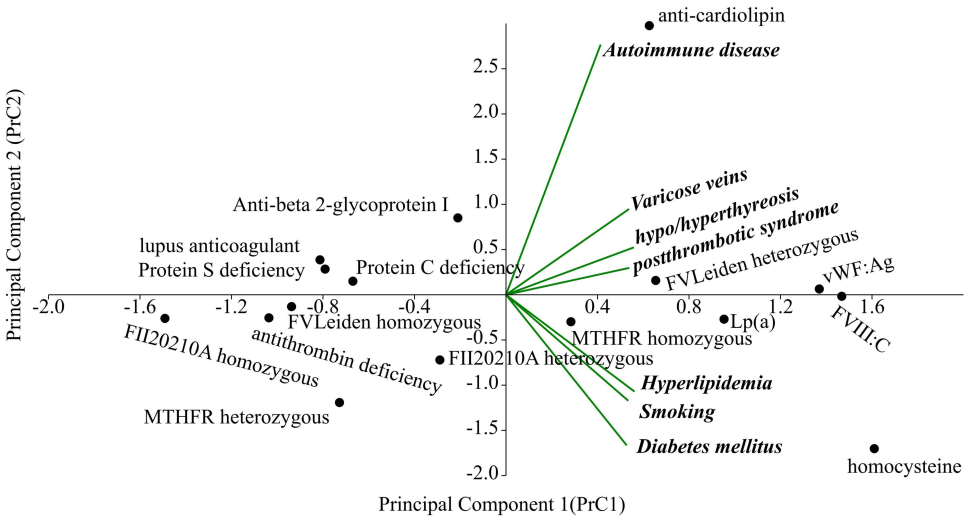

Figure 1 Relationship of thrombophilic factors and comorbidities/condition.
Abbreviations: FII20210A, prothrombin G20210A polymorphism; FVLeiden, factor V Leiden p.R2506Q mutation; FVIII:C, factor VIII clotting activity; Lp(a), lipoprotein (a); vWF:Ag, von Willebrand factor antigen; MTHFR, methylenetetrahydrofolate reductase C677T polymorphism; PrC1, Principal Component 2; PrC2, Principal Component 2.

In Figure 1 the green vectors present correlations of each comorbidity/condition with a given PrC. The length of a green vector represents the strength of the correlation between each component and comorbidity/condition. The direction of a green vector represents the (negative or positive) sign of the correlation. For example, autoimmune disease positively correlates with both PrCs, diabetes mellitus negatively correlates with PrC2, but positively correlates with PrC1. Moreover, varicose veins, postthrombotic syndrome and hypo/hyperthyreosis were mainly related to PrC1. This means if a thrombophilic lab factor (like FVIII:C) has a high value on the PrC1, it is strongly related to varicose veins, postthrombotic syndrome and hypo/hyperthyreosis as well.

Principal Component Analysis can also capture cluster structures. PrC2 separates autoimmune disease from hyperlipidemia, smoking and diabetes mellitus. Hyperlipidemia, smoking, diabetes mellitus belong to the same cluster as their vectors are close to each other (this also means that they are positively correlated) and are also strongly influenced by Lp(a). PrC1 is correlated mostly with varicose veins, postthrombotic syndrome and hypo/hyperthyreosis and they are strongly related to FVLeiden heterozygous, elevated FVIII:C, vWF:Ag and homocysteine. The laboratory risk factors can simply be represented by the PrC2 and related to the comorbidities/conditions. Hence, we can observe that aB2GPI and aCL are positively correlated with autoimmune disease, while FII heterozygous and MTHFR heterozygous are negatively correlated with autoimmune disease. PS deficiency, PC deficiency, AT deficiency, FVLeiden homozygous and lupus anticoagulant are also negatively correlated with varicose veins, postthrombotic syndrome and hypo/hyperthyreosis.

Both the highest values of Kulczynski indices (0.592 and 0.508) were associated with strong relationships: the one between "hyperlipidemia" and homocysteine, and the other between autoimmune disease and aCL. However, hyperlipidemia also had a clear relation to elevated levels of FVIII:C and vWF:Ag as well (Figure 1).

The second Principal Component Analysis involved the study of the presence of thrombophilic lab factors in connection with various locations of VTE (Figure 2). The interpretation of the results was similar to the first Principal Component Analysis with comorbidities. The localizations of thromboses can also be clustered into four groups: 1: splanchnic vein thrombosis and cerebral vein sinus thrombosis; 2: PE and PE+DVT; 3: proximal DVT, distal DVT and CRVO; 4: upper extremity DVT. Component 2 separates PE, PE+DVT, splanchnic vein thrombosis and cerebral vein sinus thrombosis from the others. This is due to PS deficiency, MTHFR heterozygous, FVLeiden homozygous, aB2GPI, AT deficiency factors which are connected to cerebral vein sinus thrombosis and splanchnic vein thrombosis. On the other hand, proximal DVT, distal DVT and CRVO are strongly connected to FVIII:C, vWF:Ag and homocysteine. PE and PE+DVT are mainly related to MTHFR homozygous. Upper extremity DVT is strongly connected to Lp(a) and FVLeiden heterozygous, PC deficiency, aCL factors. To summarize, it can be stated that FVIII:C, vWF:Ag,

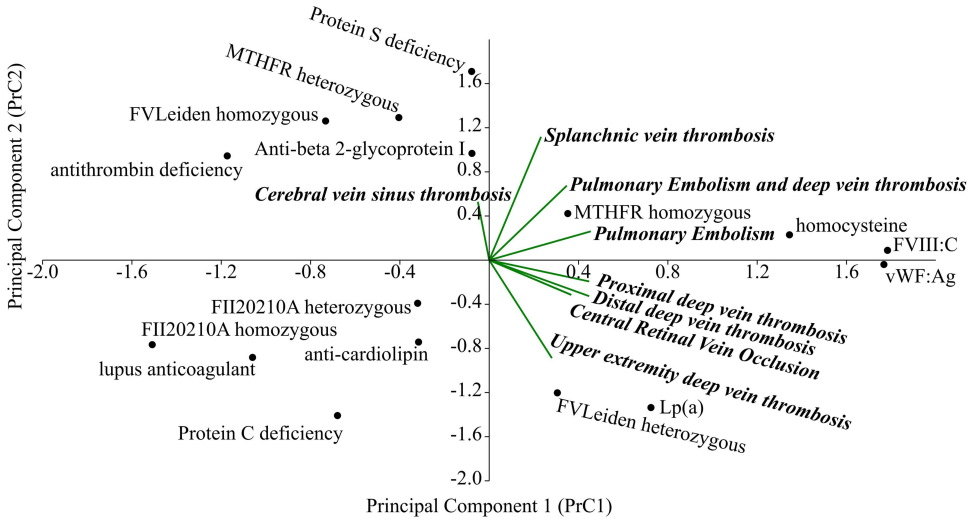

Figure 2 Relationship of thrombophilic factors and different types of thromboses.
Abbreviations: FII20210A, prothrombin G20210A polymorphism; FVLeiden, factor V Leiden p.R2506Q mutation; FVIII:C, factor VIII clotting activity; Lp(a), lipoprotein (a); vWF:Ag, von Willebrand factor antigen; MTHFR, methylenetetrahydrofolate reductase C677T polymorphism; PrC1, Principal Component 1; PrC2, Principal Component 2.

homocysteine, MTHFR heterozygous, Lp(a), aCL, FVLeiden homozygous and heterozygous, PS deficiency and PC deficiency factors had the strongest relationship with thromboembolic conditions in our study population with VTE.

In the third comparison (Table 3), we created 6 groups of VTE patients (n=266) according to the mildness and severity of the laboratory factors focusing on the importance of mild ones. MF1 is for patients who have only 1 mild lab factor, MF2 is for patients who have 2, and MF3 is for those who have 3 or more mild factors without severe factors in these groups. SF1 is denoted patients with at least 1 serious lab factor accompanied by only 1 mild factor. SF2 is for

Table 3 Comparison of Mild and Severe Groups According to Thromboembolic Events and Thrombophilia Lab Factors

Lab Factors	VTE	Mild Groups			Severe Groups		
		MF1	MF2	MF3	SF1	SF2	SF3
FVLeiden heterozygous	Proximal DVT	0.148	0.241	0.277	–	–	–
Homocysteine		0.107*	0.176	0.228	0.071*	0.200	0.261
FVIII:C		0.000*	0.160	0.431	0.125*	0.145	0.667
MTHFR heterozygous		0.000*	0.150	0.194	–	–	–
PS deficiency		–	–	–	0.357	0.400	0.500
aCL		–	–	–	0.000*	0.111*	0.316
vWF:Ag		0.000*	0.036*	0.365	0.000*	0.000*	0.615
Lp(a)		–	–	–	0.062*	0.167	0.278
FVLeiden heterozygous	Distal DVT	0.143	0.225	0.270	–	–	–
Homocysteine		–	–	–	0.000*	0.300	0.471
FII heterozygous		–	–	–	0.000*	0.143	0.556
FVIII:C		–	–	–	0.167	0.200	0.375
Lp(a)		–	–	–	0.062*	0.167	0.278
MTHFR heterozygous		–	–	–	0.000*	0.167	0.231
PC deficiency		–	–	–	0.083*	0.083*	0.143
Lupus anticoagulant		–	–	–	0.059*	0.286	0.357
aCL		–	–	–	0.000*	0.429	0.467
aB2GPI		–	–	–	0.000*	0.167	0.545

(*Continued*)

Table 3 (Continued).

Lab Factors	VTE	Mild Groups			Severe Groups		
		MF1	MF2	MF3	SF1	SF2	SF3
vWF:Ag		–	–	–	0.000*	0.250	0.615
AT deficiency		–	–	–	0.000*	0.167	0.333
Homocysteine	CRVO	–	0.188	0.380	–	–	–
FVIII:C		–	0.107*	0.167	–	–	–
Lp(a)		–	0.125*	0.257	–	–	–
MTHFR heterozygous		–	–	–	0.000*	0.000*	0.182
Homocysteine	PE	–	–	–	0.077*	0.222	0.263
PS deficiency		–	–	–	0.188	0.400	0.375
PS deficiency	PE+DVT	–	–	–	0.154	0.250	0.255
FVLeiden homozygous	Cerebral sinus thrombosis	–	–	–	0.000*	0.000*	0.333

Note: *Not significant at 5% significance level, critical value: 0.135 (significant indices above 0.135 are unmarked).
Abbreviations: FII, prothrombin G20210A polymorphism; FVLeiden, factor V Leiden p.R2506Q mutation; FVIII,C, factor VIII clotting activity; Lp(a), lipoprotein (a); AT, antithrombin deficiency; PC, Protein C deficiency; PS, Protein S deficiency; vWF,Ag, von Willebrand factor antigen; aCL, anti-cardiolipin antibodies; aB2GPI, anti-beta-2-glycoprotein I antibodies; MTHFR, methylenetetrahydrofolate reductase C677T polymorphism; DVT, deep vein thrombosis; CRVO, central retinal vein thrombosis; PE, pulmonary embolism; PE+DVT, pulmonary embolism and deep vein thrombosis at the same time; MF1, mild 1 group; MF2, mild 2 group; MF3, mild 3 group; SF1, severe 1 group; SF2, severe 2 group; SF3, severe 3 group.

patients who also have 1 or more serious factors with 2 mild factors and SF3 is for those who have severe factor(s) with 3 or more mild ones. We also sought to find tendencies in the localization of VTE within the 6 levels, in order to measure the strength of the relationship between the lab factors and the different types of VTE. The mean of the mild group was 0.045 (95% CI: 0.038, 0.053), the standard deviation was 0.076 and we obtained 0.068 for the interquartile range, the maximum was 0.431. The mean for the severe group was 0.093 (95% CI: 0.084, 0.104), the standard deviation was 0.127 and we obtained 0.155 for the interquartile range, the maximum was 0.667. The critical value was 0.135, in case the value is greater than the critical value, it can be considered significant.

In SF1, SF2, and SF3 groups, significantly increasing trends ($p < 0.05$) of DVT were found in association with the presence of two SFs such as lupus anticoagulant or PC deficiency as well as the following MFs: elevated homocysteine, vWF:Ag, aCL, aB2GPI positivity or heterozygous MTHFR mutation. We also revealed significant increasing trend of PE +DVT at the same time in connection with PS deficiency ($p<0.05$) in these groups. In addition, there was a trend-like increasing accumulation of proximal DVT in the presence of PS deficiency, elevated levels of FVIII:C, homocysteine, aCL, vWF:Ag and Lp(a) factors (all of them were significant ($p < 0.05$)) in the severe groups. Homocysteine has proven to be a general and "robust" thrombophilic factor in our investigation as it had multiple significant relationships with several thromboembolic conditions and localizations. The FVLeiden homozygous factor had a significant trend-like relation ($p < 0.05$) with only cerebral and splanchnic thromboses.

In mild groups, a significant increasing trend ($p < 0.05$) of CRVO was found in case of elevated strike levels of homocysteine, FVIII and Lp(a). Meanwhile, there was also a significant increasing trend ($p < 0.05$) of distal DVT in mild groups in the presence of FVLeiden heterozygous factor. A significant trend-like accumulation of proximal DVT was found in connection with FVLeiden heterozygous and MTHFR heterozygous factors, elevated levels of homocysteine, FVIII:C and vWF:Ag ($p < 0.05$) in mild groups.

Discussion

The clinical and economic significance of thrombosis underlines the importance of intensive research on prothrombotic states and risk factors, which can lead to clarification of more effective prevention, diagnosis and treatment. Without a high-risk surgical intervention, active cancer, radio/chemotherapy, the risk assessment is often complex and difficult. In unselected patients with first episode of VTE, testing for inherited thrombophilia seems to have little benefit in predicting the risk of recurrence after stopping anticoagulant therapy.[39] However, thrombophilia testing might be useful in selected patients with benefits both in the evaluation of cumulative risk and antithrombotic therapy.[5,6,10,40] In this paper, we

examined the potential role of laboratory thrombophilic risk factors and comorbidities for VTE and/or ATE in the absence of serious triggers (eg, a high-risk surgical intervention, active cancer or radio/chemotherapy).

We found that patients getting their first thrombosis were younger in VTE than in ATE group, which can be due to the different pathomechanism of VTE and ATE. VTE is more likely to be associated with inherited/acquired thrombophilic lab factors causing thrombosis in younger ages, while ATE mostly develops on the basis of atherosclerosis which correlates with age.[41] However, thrombophilic factors, comorbidities and conditions studied in the present paper were much more common both in our VTE and ATE groups as compared to the general population or in unselected patients with thrombosis. As expected, we found that smoking was more common in ATE (as a classic risk of atherosclerosis), but recurrent events, provoking factors, varicose veins and postthrombotic syndrome were more frequent in the VTE group. FVIII:C was more frequently present in VTE than in ATE group, suggesting its more important role in the pathomechanism of VTE (irodalom!).

Smoking and diabetes are frequently associated with lipid abnormalities. Unsurprisingly, in our study we also found their strong positive correlation with hyperlipidemia which is significantly associated with elevated Lp(a), as well.[1,42] Varicose veins with postthrombotic syndrome were strongly related to several "mild" lab factors in the present study population, drawing attention to the potential role of these factors in VTE complications.[14,23,25] It is well known that aB2GPI and aCL factors are associated with autoimmune diseases,[13] which could be confirmed by our investigations, as well. Moreover, postthrombotic syndrome is negatively correlated with FVLeiden homozygous mutation, lupus anticoagulant, AT, PC or PS deficiency. Patients with these "severe" factors are generally on prolonged anticoagulation therapy; therefore they have less chance to develop postthrombotic complication. Varicose veins are also negatively correlated with severe factors; the coincidence of varicose veins with intense anticoagulant treatment was less common. Negative correlation was also observed among hypo/hyperthyreosis and these "severe" factors. This latter negative association might be a consequence of a low frequency of these diseases compared to several other comorbidities in the present study population. Upper extremity DVT was related to Lp(a), FVLeiden heterozygous mutation, aCL and PC deficiency in our patients. Among these less frequent, although serious thrombosis, the splanchnic vein and the cerebral vein sinus thromboses seemed to be strongly connected to some severe factors such as PS and AT deficiency, FVLeiden homozygous mutation, showing essential role of severe thrombophilic factors in these unusual site thrombosis.[5] But we interestingly found similar connections also with MTHFR heterozygous and aB2GPI in our patients, suggesting these factors can add supplementary risks for manifestation of thrombosis in these severe localizations. Other more common factors such as FVIII:C, vWF:Ag, moreover, the elevated homocysteine level connected to MTHFR heterozygous mutation are strongly associated with proximal and distal DVT which might have a significant relationship with impaired peripheral venous circulation. A significant trend-like accumulation of proximal DVT was found in connection with FV Leiden heterozygous mutation as a frequent laboratory finding in Hungarian population.[22] PE and PE+DVT at the same time are related to MTHFR homozygous mutation in our sample as shown in an earlier study.[43]

We investigated trends among different types of VTE and the severe/mild thrombophilic factors. Significant increasing trends of VTE (proximal/distal DVT, PE, PE+DVT, CRVO and cerebral sinus and splanchnic thromboses) were found in association with the presence of severe factors. A significant increasing trend of VTE (proximal/distal DVT or CRVO) was found together with the accumulation of mild factors suggesting their similar significant role in the pathomechanism of VT.

To summarize, it can be stated that among our investigated laboratory thrombophilic factors, FVIII:C, vWF:Ag, Lp(a), aCL, PC deficiency, FVLeiden homozygous and elevated homocysteine and MTHFR polymorphism had the strongest connection with thromboembolic diseases in our relatively large study population in Central-Eastern Europe.

We advise some caution regarding overall interpretation of our results considering the limitations. In our sample there were significantly more women than men. The sample was heterogeneous; the patients suffered from different thrombotic issues complicated with several other diseases and risk factors. The relatively high number of anterior ischemic optic neuropathy/retinal artery thrombosis among ATE patients could also have influenced our results. The inclusion criteria were also different; as the laboratory screenings were made in several clinical situations, detailed previously, by the decisions of the clinicians. The study population was relatively large, but all of the cases were collected in our Central-Eastern Europe Centre so results cannot be generalized to risk assessments for overall population.

Conclusion

Our results cannot provide robust evidence in favor of thrombophilia testing in general in VTE or ATE. However, in selected cases, in addition to patients' and family history, an extended thrombophilia screening (including the "mild" factors) could help the risk assessment and it can be useful in cases when the antithrombotic prophylaxis or the duration of the treatment is unclear and no serious trigger factor exists. Since the hit ratio is high, a detailed thrombophilia screening might be cost efficient in case of thrombotic diseases in a carefully selected patient population.

More studies are needed to determine the proper benefit of testing and selecting the individuals who can benefit from thrombophilia screening, in addition to achieving a personalized recommendation for estimation of the risk of thrombosis or antithrombotic prophylaxis.

Abbreviations

aB2GPI, anti-beta-2-glycoprotein I antibodies; aCL, anti-cardiolipin antibodies; AT, antithrombin; ATE, arterial thromboembolism; CRVO, central retinal vein thrombosis; DVT, deep vein thrombosis; FII, prothrombin G20210A polymorphism; FVLeiden, factor V Leiden p.R2506Q mutation; FVIII:C, factor VIII clotting activity; KMO, Kaiser-Meyer-Olkin; Lp(a), lipoprotein (a); MF, mild thrombophilic factor; MTHFR, methylenetetrahydrofolate reductase C677T polymorphism; PC, protein C; PrC, Principal Component; PE, pulmonary embolism; PE+DVT, pulmonary embolism and deep vein thrombosis at the same time; PROC, human protein C gene; PROS1, human protein S gene; PS, protein S; SF, severe thrombophilic factor; VTE, venous thromboembolism; vWF:Ag, von Willebrand factor antigen.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

This study was conducted in accordance with the principles of the Declaration of Helsinki.

Funding

There is no funding to report.

Disclosure

The authors have no conflict of interest to declare that are relevant to the content to this article.

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